These expansions could be destabilized by adding a PI3K inhibitor, or by depleting the membrane cholesterol suggesting that the formation of these branches is due to membrane lipid domain formation around nlg-1 clusters. To confirm the role nlg-1 clustering, we have resorted to polarization fluorescence lifetime imaging microscopy to spatially resolve the nlg-1 oligomerization state at different location through out these expansions. We have exploited the photophysical properties of cerulean, a fluorescent protein, to assess the interneuroligin distances and decipher nlg-1 molecular interactions. These measurements confirmed that neuroligin tight clustering was involved in the formation of membrane cholesterol rich domains enabling the recruitment of PI3K, which in turns promotes the growth and the maintenance of these expansions.

3584-Pos

Don't Fence Me in: Evidence for a 'fence' that Impedes the Diffusion of PIP_2 Into and Out of Nascent Phagosomes in Macrophages

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To account for the many roles that phosphatidylinositol 4,5-bisphosphate (PIP_2) plays (e.g., in phagocytosis, exocytosis, activation of ion channels) a number of investigators have suggested there are separate pools of PIP_2 in the plasma membrane. Recent experiments show that the free concentration of PIP_2 is indeed enhanced in nascent phagosomes, syntaxin clusters, and the furrows of dividing cells. Kinases that produce PIP_2 (PIPkins) are also concentrated in these regions. But how is the PIP_2 produced by these PIPkins prevented from diffusing rapidly away? *First*, proteins could act as 'fences/corrals'around the perimeter of these regions. *Second*, some factor (e.g., a protein that acted as a PIP_2 buffer) could decrease significantly the diffusion coefficient, D, of PIP_2 within these regions. We used FCS and FRAP to investigate these two possibilities in the nascent phagosomes of J774 macrophages injected with fluorescent PIP_2. FCS measurements show PIP_2 diffuses with similar fast diffusion coefficients in the nascent phagosomes and in the bulk (unengaged) plasma membrane: $D = 0.6 \pm 0.3\ \mu m^2/s$ and $0.8 \pm 0.2\ \mu m^2/s$, respectively. FRAP measurements show the fluorescence from PIP_2 recovers slowly (>100 s) after photobleaching the entire nascent phagosome but recovers rapidly (<10 s) in a comparable area of the plasma membrane outside the cup. These results support the first hypothesis: a 'fence'impedes the diffusion of PIP_2 into and out of nascent phagosomes. The nature of the PIP_2 fence remains an enigma: although actin filaments are concentrated at the perimeter of the forming phagosomes, electrostatic and Brownian Dynamics calculations suggest individual negatively charged actin filaments near the membrane do not significantly impede the diffusion of PIP_2 into or out of the cup.

3585-Pos

Hard to Fence You in: Computational Approaches to Explore the Hypothesis that Actin Filaments Impede PIP2 Diffusion in Membranes

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Experiments described by Golebiewska et al. at this meeting suggest the existence of a 'fence'that impedes the diffusion of phosphatidylinositol 4,5-bisphosphate (PIP2) into and out of nascent phagosomes in macrophages. Although the nature of the fence remains an enigma, actin filaments are plausible components. They are highly negatively charged (as is PIP2), are swept away from the central region and are concentrated at the perimeter of the forming phagosome. To explore the actin fence hypothesis, we have used (1) Poisson-Boltzmann continuum electrostatics and a grid-based repulsive potential to describe a fence model made of a single layer of actin filaments, and (2) Brownian dynamics to describe the diffusion of PIP2 molecules modeled as single spheres. The simulations with actin filaments positioned parallel to the membrane indicate that a single filament without attached proteins does not significantly impede the diffusion of PIP2. A helical stripe of basic residues on the acidic actin filament provides a hole in the putative fence through which PIP2 can diffuse, no matter how close the filament is positioned to the membrane. Results from simulations of PIP2 diffusion out of corrals formed of multiple layers of actin filaments, and mazes of non-electrostatic barriers will also be presented.

3586-Pos

Regions of Correlated Fluctuations in Membrane Lipid Concentrations as a Consequence of Charged Cytoplasmic Lipids

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The cytoplasmic leaflet of the mammalian plasma membrane is characterized by charged lipids such as phosphatidylserine and phosphatidylinositol and its phosphorylated derivatives PIP and PIP2. Their concentrations vary from about ten to less than one percent. The charges are balanced by counter ions in the cytosol. As a consequence there are electric dipole moments in the cytoplasmic leaflet. The extra-cellular leaflet has essentially no charged lipids. In contrast to the electric dipole moments of the lipid head groups in both leaves which, due to their opposite orientation, essentially cancel one another at large distances, the dipole moments of the charged lipids in the cytoplasmic leaf interact via a long-ranged force. As a consequence, the fluctuations in density of these dipole moments are characterized by a non-zero length which depends upon the dipole density and temperature. Because the tails of these lipids couple them to the lipids of the extra-cellular leaflet, composition fluctuations in the outer leaflet will also display characteristic sizes. Thus the presence of charged lipids in the cytoplasmic leaflet results in coupled fluctuations in both leaves of a characteristic size. Such coupled regions could be important in the signaling processes which are associated with the charged lipids.

3587-Pos

Probing Spatial Organization in Cell Membrane at the Immunological Synapse

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The large-scale spatial arrangement of cell surface molecules has been gradually realized to regulate specific cellular outcomes in many cellular processes. This phenomenon is particularly striking in the antigen recognition by T cells. Signaling through discrete T cell receptors (TCRs) in the context of immunological synapse, involves the orchestrated movement and reorganization of TCRs on multi length scales. Microcluster movement is believed to be associated with centripetal actin flow, but the underlying physical mechanism remains unclear. By using the hybrid live T cells-supported membrane system, our study to probe the membrane spatial organization of T cells at their immunological synapse and its connection with TCRs movement will be discussed.

3588-Pos

The Effects of High Voltages on the Morphology of a Dppc Lipid Bilayer

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Biological membranes are often subject to large voltages compared to their small thickness, especially true in nerves where fluxes of ions and corresponding voltage changes are thought to be the main mechanism behind the nerve signal. Yet the effects that these voltages have on the phospholipids that makes up the membrane are largely unknown. Lipids of biological membranes are often charged or swittterionic, high electrical fields should be expected to have a large effect on their organization and thermodynamical properties. Fluorescence microscopy is utilized to image the effects of high voltage fields on the domain structure of a model system consisting of a Langmuir-Blodgett monolayer of phospholipids.

3589-Pos

Lysophosphatidic Acid Interactions with Model Membranes: a Novel Cell Signaling Regulatory Mechanism?

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Lysophosphatidic acid (LPA), the structurally simplest of the glycerophospholipids, is a potent second messenger whose functional diversity makes it a compelling target in lipid research. LPA, the effects of which include cell motility and proliferation, platelet activation, fertility and development, and neuropathic pain, is believed to act through a family of G protein-coupled receptors (GPCR). Since some members of this family of proteins are localized in ordered lipid domains (membrane rafts), a role for LPA in altering and re-ordering membranes as part of regulation of the signaling pathway cannot be discounted.
As part of a series of efforts to obtain biophysical information about the effects of LPA on membranes, we have employed the Langmuir monolayer technique and isothermal titration calorimetry (ITC) to measure the kinetics and thermodynamics of LPA intercalation into lipid films and bilayers of various compositions representing different physical phases known to exist in biomembranes.

Our results indicate that LPA-membrane interactions depend on initial surface pressure, phospholipid headgroup and degree of acyl chain saturation, presence and amount of cholesterol, aqueous media conditions, and aggregation state of LPA. These data suggest that, in addition to its function as a ligand for specific GPCR, LPA interacts directly with the target membrane, constituting a role for this phospholipid as a physical regulatory molecule for LPA cellular signaling pathways.

3590-Pos

Molecular Hydration Investigated using Extended Membrane Surfaces

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In biological cells and in solutions, biomolecules are in constant competition for water. The availability of water is critical to a wide array of processes such as protein folding, molecular recognition, and cell signaling. To understand these mechanisms, we distinguish between the water molecules residing in the vicinity of biological macromolecules and the most distant water molecules in the bath. What happens on the surface of macromolecules? How to investigate the role and the properties of interfacial water? The problem is that the spatial extent of hydration layers is very small, on the order of nanometers or less. How to produce an experimentally measurable signal? Our approach to investigate the many remaining mysteries of molecular hydration is by using the naturally extended water surfaces within multilamellar lipid structures. We show how addition of salts, sugars, and most buffers make the interlamellar (D) spacing of synthetic phospholipids membranes to increase. In contrast to this behavior, low concentrations of highly hygroscopic molecules such as PEG (polyethylene glycol) and DMSO (dimethyl sulfide) are found to decrease the D-spacing. A very interesting case is that of the small molecular weight PEG 400 with unusual effects on the interfacial hydration, in such a way that at some concentration threshold the PEG molecules overcome a mixing barrier and become included in the forbidding interlamellar water space. This behavior is due to competitions between the strong exclusions forces from extended hydration surfaces and entropy. We are currently investigating how biological relevant molecules such as ''Factor V'' (BSA Bovine Serum Albumin) are modifying cellular osmoregulation and its effects on membranes stress. Knowing how molecules interact at the lipid-water interface could prove beneficial in the drug design of anesthetics, cryoprotectants of mammalian cells, and in general, of molecular stressor affecting biological cells.

Signaling & Membrane Transformations

3591-Pos

Determination of Threshold Forces for Tether Formation in Vesicles

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Membrane tether experiments, which involve applying point forces to membranes to form thin tubes of the membrane, provide a powerful method to drastically alter membrane curvature. To study the formation of membrane tethers, we have designed and built a magnetic force transducer (MFT) using microfabrication techniques. MFTs have traditionally suffered from an inability to precisely calibrate the force. Quantitative determination of the tether formation force using theoretical models of membrane mechanics, may be done very accurately if the applied force is known with sub-piconewton accuracy. Our initial results indicate our ability to control the amplitude of the force up to 10 pN within +/- 0.2 pN over a constant length of 50 microns. We have used this device to determine tether formation forces from POPC giant unilamellar vesicles and find that formation forces range from 3 to 10 pN. When tethers are repeatedly pulled from the same vesicle, the formation force is constant, suggesting that individual GUVs may have differences in their mechanical properties.
A particular advantage of our device is that a wide range of dynamic force profiles can be applied via a computer-controlled interface, enabling studies of responses of membranes to dynamic force application at kilohertz frequencies.

3592-Pos

Quantifying Pathogen Recognition of the Cell Membrane: Simple Sugars Simulate the Functions of Complex Glycans

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The first step leading to the breaching of the cell membrane and infection of healthy cells is often the multivalent recognition and adhesion of glycan binding proteins (GBPs) on pathogens and glycans on host cell membranes. Such multivalent interaction depends critically on the mobility and density of signaling molecules on the membrane surface. While glycan microarrays have been used in exploring multivalent interactions, the lack of mobility and the difficulty in controlling surface density both limit their quantitative applications. Here we apply a fluidic glycan microarray, with glycan density varying for orders of magnitude, to profile cell surface interaction using a model system, the adhesion of Escherichia coli (E. coli) to mannose. We show the quantitative determination of monovalent and multivalent adhesion channels; the latter can be inhibited by nanopartices presenting a high density of mannosyl groups. These results reveal a new E. coli adhesion mechanism: the switching in the FimH adhesion protein avidity from monovalent to multivalent as the density of mobile mannosyl groups increases; such avidity switching enhances binding affinity and triggers multiple fimbriae anchoring. Affinity enhancement towards FimH has only been observed before for oligo-mannose due to the turn on of secondary interactions outside the mannose binding pocket. We suggest that the new mechanism revealed by the fluidic microarray is of general significance to cell surface interactions: the dynamic clustering of simple sugar groups (homogeneous or heterogeneous) on the fluidic membrane surface may simulate the functions of complex glycan molecules.

3593-Pos

Dynamical Basis of the Enhancement of the Enzymatic Activity of Factor VIIa by Tissue Factor

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Activation of coagulation factors is regulated by their binding to and complex formation on the surface of anionic membrane resulting in an increase of the enzymatic activity by several orders of magnitude. Complex formation of tissue factor (TF) and factor VIIa (FVIIa) on anionic membranes constitutes one of the key steps in the coagulation cascade. The mechanisms for the enhancement of the enzymatic activity of FVIIa by TF is not fully understood, primarily due to the lack of atomic models for the membrane-bound form of the TF:FVIIa complex.
We report a first membrane-bound model of the TF:FVIIa complex resulting from large-scale molecular dynamics simulations. The dynamics of FVIIa and soluble TF (sTF) was investigated in solution and on the membrane, both in their isolated and complex forms. Our model of membrane-bound GLA domain was used to construct the membrane-bond models of the TF:FVIIa complex and monomers. All-atom simulations were performed for tens of nanoseconds to investigate the protein dynamics after establishing optimal protein-protein/protein-lipid interactions.
The results reveal that sTF restricts the motion of FVIIa, thus optimally orienting its catalytic triad for the interaction with its substrate factor X (FX). Several direct interactions between the membrane lipids and the side chains of sTF, including the regions of K159-K166 and D180-N184 that form the exosite of the substrate FX, are observed. Interestingly, sTF sustains two distinct orientations against the membrane and different conformations of the K159-K166 loop, in the isolated and complex forms. These findings suggest that TF orients itself on the membrane surface through the interactions both to the membrane and FVIIa, independent of its trans-membrane anchoring helix and that the exosite on TF is available for FX binding only after TF:FVIIa complex is formed.

3594-Pos

Regulation of Phospholipase C Beta - Rac1 Cytoskeletal Pathways by Gamma Synuclein

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The breast cancer specific gene protein 1 also known as γ-synuclein is undetectable in normal or benign breast lesions, but it is highly expressed in infiltrating breast cancer. The precise role of γ-synuclein in malignancies is not well known. We investigated whether γ-synuclein might affect activity of phospholipase Cβ2 (PLCβ2). PLCβ2 is also absent in normal breast tissue, but it is highly expressed in breast tumors where it is correlated with the progression and migration of the tumor. Expression of PLCβ2 is highly correlated with expression of g-synuclein. We found that γ-synuclein binds PLCβ2 in vitro with high affinity, Kd = 23 +/- 3 nM. PLCβ2 is activated by heterotrimeric G protein and by members of Rho family of GTPases (in particular Rac1), which are